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U1S S1068 S1076 S1080 S1081 S1304 S1310 S1364

(56) Documents cited
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(58) Field of search
UK CL (Edition K) C3U UDB UDE
INT CL[5] C08B
On-line databases – W.P.I.

(54) Gel production from plant matter

(57) A method of producing a gel material comprises firstly providing an aqueous soluble hemicellulosic starting medium which is free of glucans and obtainable from testaceous plant material. The starting medium is then extracted with a non-acidic reagent and reacted with an oxidising system comprising at least one peroxide together with at least one oxygenase (such as a peroxidase).

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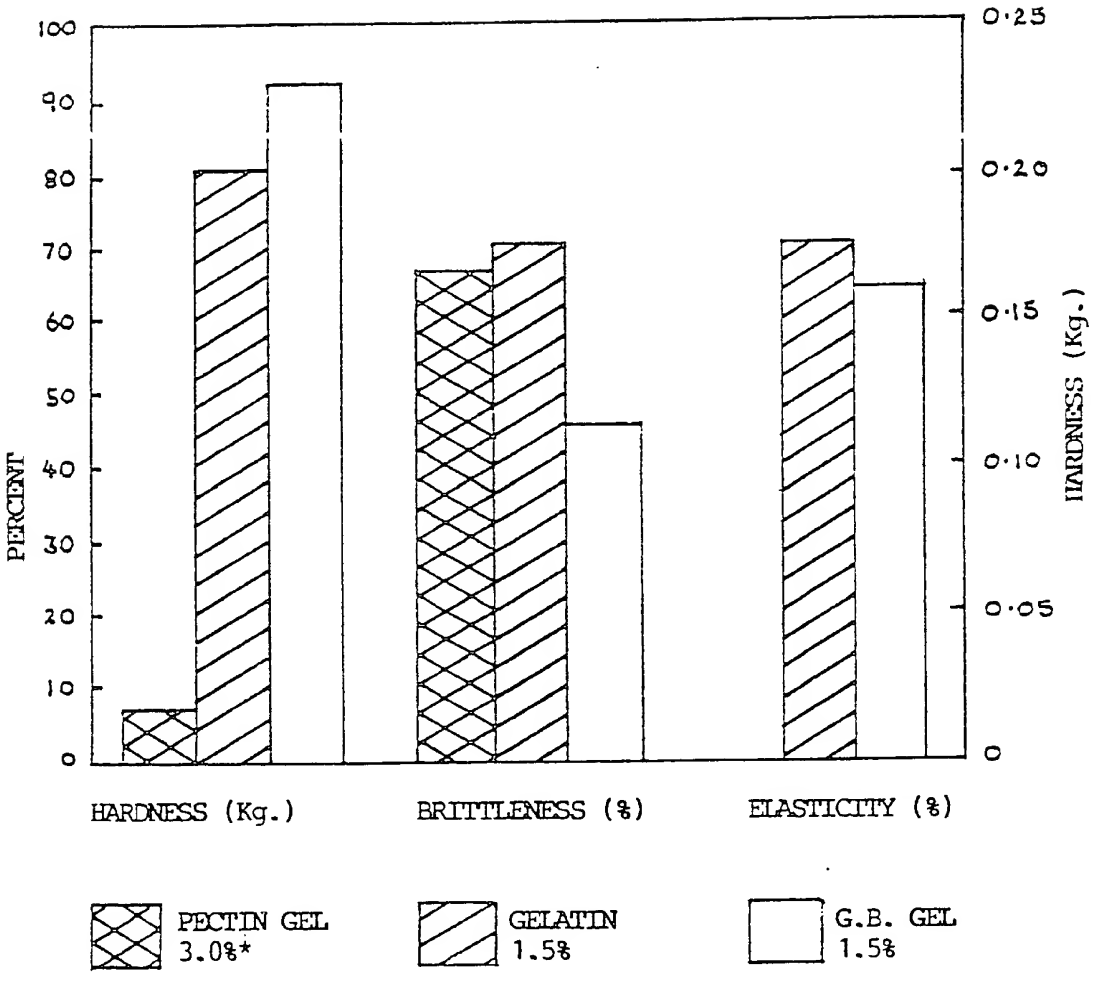
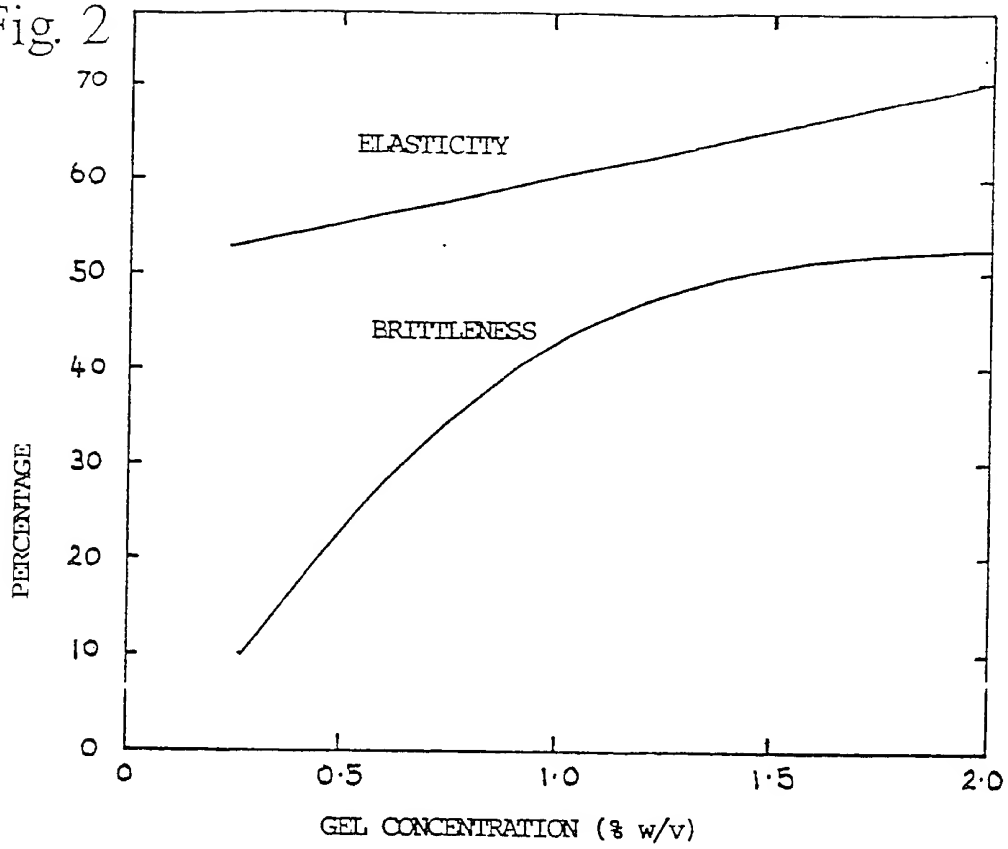
At least one drawing originally filed was informal and the print reproduced here is taken from a later filed formal copy.

Figure 1



* PECTIN GEL FROM FRENCH PATENT 2545101

Fig. 2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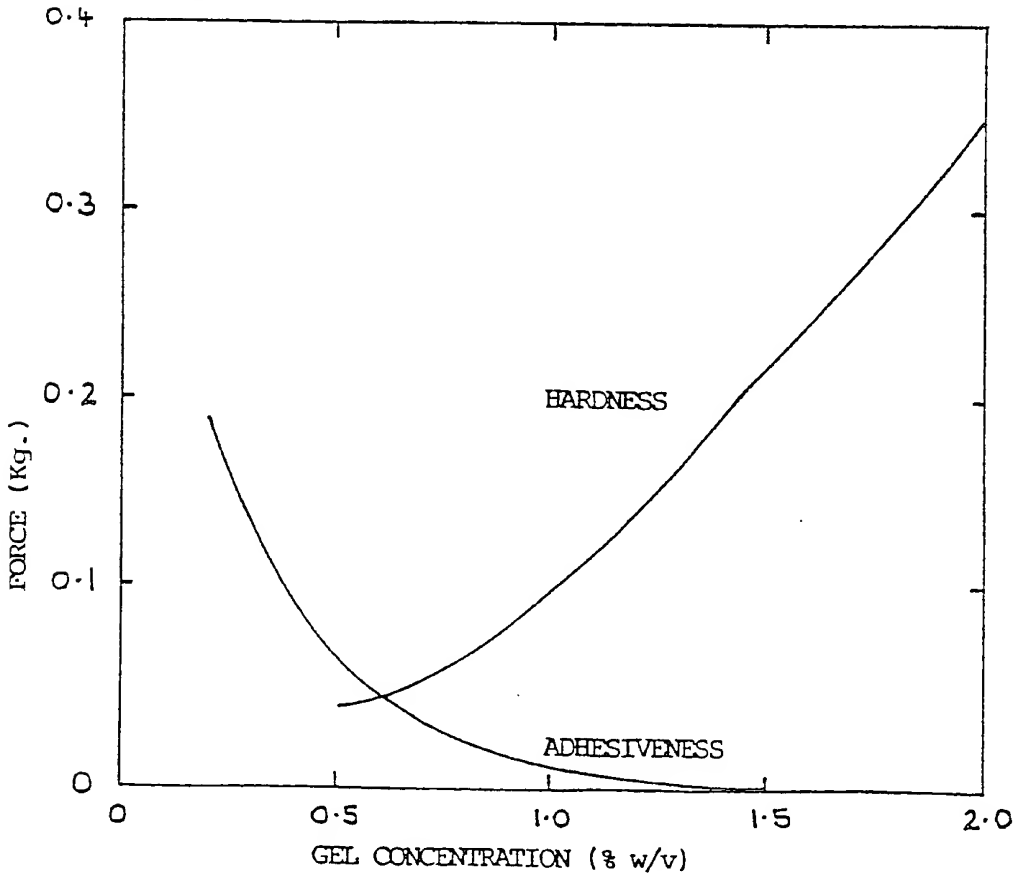
60
50
40
30
20
10
0
PERCENTAGE
ELASTICITY
BRITTLENESS
0
0·5
1·0
1·5
2·0
GEL CONCENTRATION (% w/v)

Fig. 3


0·4
0·3
0·2
0·1
0
FORCE (Kg.)
HARDNESS
ADHESIVENESS
0
0·5
1·0
1·5
2·0
GEL CONCENTRATION (% w/v)

Gel Production from Plant Matter

The present invention is concerned with the production of gels from plant matter and the resulting gels.

Large numbers of plant sources contain hemicelluloses, which are composed of various arrangements of pentoses (such as xylose and arabinose), hexoses (such as mannose, glucose and galactose) and/or uronic acids (such as glucuronic and galacturonic acid). Examples of hemicellulosic materials include xylans (such as arabinoxylan), mannans and galactans, which may be substituted by phenolic acid residues such as ferulic acid (4-hydroxy-3-methoxycinnamic acid), coumaric acid (p-hydroxycinnamic acid) or vanillic acid (4-hydroxy-3-methoxyl benzoic acid). Such materials occur naturally in cereals such as maize, barley (including malted barley), wheat, oats and rice; pulses, such as soya; legumes and fruit.

French patent specification 2545101 is concerned with modification of sugar beet pectins by reacting an oxidising system comprising an enzyme (such as peroxidase) and an oxidising agent (such as hydrogen peroxide) with pectins which have been isolated from sugar beet. The isolation of pectin comprises subjecting the sugar beet to acidic extraction and heat treatment.

According to the present invention, there is provided a method of producing a gel material, which method comprises:

(a) providing an aqueous soluble hemicellulosic starting medium which is substantially free of glucans and is obtainable from testaceous plant material;

(b) extracting said starting medium with an alkaline reagent; and

(c) reacting the extracted material with an oxidising system comprising at least one peroxide, together with at least one oxygenase (such as a peroxidase).

The soluble hemicellulosic starting medium is typically prepared from waste testaceous plant material containing a significant quantity (such as at least about 20%) of arabinoxylan or glucuronoarabinoxylan, which is present in nature primarily in the cell wall regions. Examples of preferred such sources include waste materials which are rich in cell walls, such as cereal husk or bran, or legumes (pulses). Typical cereal husk or bran includes maize, barley, wheat, rice or oats, or malt or malt culms (dried germinated barley rootlets).

In a preferred embodiment, the hemicellulosic starting medium is in a substantially ground form having a particle size of not more than about 100 microns. The plant material is therefore typically ground, either in dry or wet form (such as milling or wet grinding known as maceration) to the required particle size The ground material is typically air classified or sieved to remove starch. The method may comprise starch removal by suitable enzyme treatment, for example, with diatase (alpha and/or beta-amylase).

The glucans are preferably removed from the plant material by enzyme digestion with carbohydrase enzymes such as glucanase.

The insoluble enzyme treated material may then be dried (in air) before further processing. The plant material may have been pre-treated so as to remove the glucans prior to application of the present method, but it is preferred that the method according to the invention involves enzyme treatment so as to remove glucans following the above described grinding of the plant material.

Suitable glucanases for use according to the invention are commercially available under the trade marks Viscozyme, Biofeed and Biofeed Plus which typically also have hemicellulase, cellulase, arabinase and xylanase activity. Viscozyme is currently preferred.

The alkaline extraction preferably comprises treatment with hot water or weak alkali (less than 0.5%). Preferred alkalies are NaOH and KOH. The alkali is preferably used in an amount of 0.1 to 10% (typically 0.5 to 2.5%), for times of from 20 minutes to 5 hours (typically about 2 hours). Alternatively, gels may be produced from wheat bran and barley dust or culms by using hot water in place of alkali.

The alkaline extraction may be at a temperature of from 30 to 100°C and is typically at a temperature of 60 to 90°C, generally for 10 minutes to 5 hours. For strong gels, temperatures of 60 to 75°C are preferably used for 0.5 to 1.5 hours; for weaker gels temperatures of 60 to 85°C are preferably used for 2 to 5 hours. Hot water extraction is carried out at temperatures of 50 to 80°C (typically 60 to 70°C) for 0.5 to 2 hours (typically 1 to 1.5 hours). The extraction is generally effected with gentle stirring. The resulting extracted material generally comprises insoluble cellulose and soluble hemicelluloses; the cellulose is typically removed by centrifugation, either with or without acidification.

It is advantageous to avoid extreme conditions (such as sustained contact of the hemicellulosic medium with sodium hydroxide or temperatures above the above-described preferred range) during alkaline extraction in order to optimise the gelling characteristics of gel material produced by a method according to the present invention.

Alkaline extracting will produce an extracted material substantially free of pectins as the latter are labile in alkaline conditions and are extractable by acidic reagents as described in FR 2545101.

Following alkaline extraction the hemicellulosic material, which is rich in arabinoxylans and is substituted by phenolic acids, is preferably neutralised (for example, using hydrochloric, sulphuric, acetic or citric acid, of which citric acid is preferred). Neutralisation is advantageous in that it helps to preclude rapid hydrolysis of ferulic acid residues present in the extracted material; such hydrolysis would damage the gelling properties of the material. The solids can be removed from the neutralised extract by filtration or centrifugation which results in improved gel properties.

Purification of the hemicellulosic material may then be carried out by precipitation with an alcohol such as methanol or ethanol (or industrial methylated spirit), or iso-propanol (propan-2-ol). Such alcohols may be added in amounts of from 1.5 to 3.5 volumes according to the fraction desired by molecular weight. The hemicellulosic material may alternatively be purified by passage through an activated carbon column and subsequently concentrated by precipitation with ammonium sulphate at 70-80% saturation or any of the above alcohols used for precipitation. Alternatively the concentration of the eluate may involve drying (such spray or vacuum rotary drying) and redissolving of the eluate.

The hemicellulosic material may be further purified by ion-exchange treatment, preferably with a cation exchange resin to remove cationic impurities.

Differential precipitation at this stage can provide fractions of the polysaccharide which vary in molecular weight and exhibit different rheological properties and consequently viscoelastic properties of the gels they produce. For example precipitation with ammonium sulphate at saturations of between 60 and 80% yields fractions differing in molecular weight; similarly addition of ethanol of 1.7 to 3 volumes yields the same range of fractions.

After separation by filtration or centrifugation, and redissolving of the precipitate in water, a second precipitation may be carried out by addition of 2 to 4 volumes of alcohol. The fraction obtained may be filtered (and dried on the filter using ether) or redissolved in water and lyophilised.

The salt content may be lowered if wished (for example, if the final gel is to be used in foodstuffs), typically by dialysis or tangential flow ultrafiltration. The de-salted material may be separated as an anion exchange resin such as Purolite A500 to produce fractions differing in charge (dependent on uronic acid content). Selection of fractions at this stage can further control the rheological/viscoelastic properties of the final product. The resulting material may be dried (for example, by spray drying, freeze drying, vacuum rotary drying or drying on a filter using diethyl ether) at this stage; the resulting dried material may be rehydrated prior to treatment with an oxidising system as described below.

The rehydrated material (or, if relevant, the non-dried material) is then treated with a peroxide (such as H_2O_2) and a peroxidase (such as horseradish peroxidase). By varying the hydrogen peroxide concentration, and hence the number of free ferulic acid groups that become di-ferulic cross links, the extent of cross-linking within the resulting gel can be controlled. For example, a 0.5% solution of the hemicellulosic starting medium may produce gels with "hardness" varying from 0.008kg to 0.058kg by adjusting the concentration of hydrogen peroxide in the enzymic reaction. The term "hardness" is a measure of the viscoelastic properties of the gel.

The gel properties may be further modified by the conditions used in peroxidase treatment. The treatment with a peroxidase (with a small amount of the peroxide) can result in a weak to strong clear gel at concentrations of 0.05 to 10% (preferably 0.5 to 2.5%). The balance is generally water. Polyvalent metal cations (such as Ca^{2+}, Cu^{2+}, Zn^{2+}, Fe^{3+} or Al^{3+}) added prior to peroxide/peroxidase treatment will modify the gels, for example such that they can subsequently break into sols.

In any case, the resulting gel, which is constituted of cross-linked fibrous material comprising phenolic acid substituted arabinoxylans, is highly thermostable and may be autoclaved. (For example, the gel may withstand 15 psi at 122°C for 15 minutes). The purified gels in particular can be made with reproducible viscoelastic and rheological properties.

Further control over the viscoelastic properties (such as brittleness) may be exercised by addition of sugar, salts or alcohols, or by treatment with carbohydrase enzymes.

The peroxidase is typically used in an amount of 1 to 100 micrograms per gram of substrate; the peroxide is typically used in an amount of the order of one tenth of the amount of peroxidase.

Viscous solutions rather than gels can be produced by either further limitation of the peroxide concentration or by using a solution having a hemicellulosic concentration below the critical gel-forming concentration of about 0.05%. For example, solutions of viscosity varying between 100 and 500 cP may be produced from an 0.1% hemicellulosic concentration by limiting the peroxide concentration to levels below those which form gels.

The extract may co-gel with other hemicellulosic-derived materials in such a way that the two gelling agents are synergistic. For example, extract material derived from maize in the method according to the invention may be blended with extract material derived from other cereals (such as wheat, malt or barley) in the method according to the invention, in proportions in which neither would form a firm gel alone, but a firm gel is formed with the two materials. For example, a firm gel can be obtained with 0.7 to 3% of material derived from maize and about 2% of material derived from wheat (all the above proportions being on a solids basis).

The gel material according to the invention may have a wide variety of uses, of which the following are exemplary:

1. In medicinal compositions for example as a topical formulation (such as for treatment of burns), as a carrier for iron or zinc, a thickener for parenteral compositions, or an encapsulating agent, or as a slow release vehicle for drug delivery (either for oral, parenteral or anal delivery).

2. In foodstuffs or animal feeds, for example, as a stabiliser for ice cream or the like, as a suspending agent for particles such as coconut, as a glazing agent for meat or the like, as a setting agent for jams, or a thickening agent for gravies, purees, sweets, soups or the like.

3. In the oil industry, for example, for sealing strata above oil deposits, as an additive to drilling muds or the like, and recovery of oil from oil-bearing strata.

4. In the microbiological industry, for example as a gelling agent, a spore biocontainer or a culture biocontainer.

5. In the agricultural industry, as a slow release pesticide biocontainer, a plant culture medium, an anti-drying agent or the like.

Gels obtained according to the invention may be prepared such that they eventually break down to the sol form.

The present invention is further illustrated by reference to the following Examples and accompanying diagrams which do not limit the scope of the invention in any way.

EXAMPLE 1

Production of a Firm Gel from Corn (Zea Mays)

1. Grinding

Corn bran was subjected to grinding which involved initial wet milling followed by dry milling to an average particle size in the range 80-300 microns.

2. Enzyme Treatment

0.01% w/w of a cytase enzyme at 45°C for 2 to 24 hours depending on raw material type and textures (e.g. for milled corn bran a period of about 6 hours).

3. Alkali Extraction

A 10% (w/v) suspension of the milled corn bran in 1% w/v potassium hydroxide (aqueous) was prepared and gently stirred at 65° - 80°C for 2-3 hours.

4. Separation

The insoluble material, consisting mainly of cellulose, was removed by centrifugation at 2500 rpm.

5. Neutralisation/Dialysis

The supernatant was carefully decanted, neutralised with hydrochloric acid (or citric acid) and dialysed against running tap water for 2 days.

6. Gelling

The concentration of the dialysed extract was adjusted to 3% w/v with deionised water. 100ml of this solution was taken and 1ml of 100 micrograms/ml horseradish peroxidase mixed in thoroughly. When distributed, 0.5ml of hydrogen peroxide at 40 micrograms H_2O_2/ml was added and mixed in; the mixture was then left to set at ambient temperature (5-15 min) or at a higher temperature (1-2 min at 40°C).

An Instron Texture Profile Analyser was used to measure the hardness, brittleness and elasticity of the following:- a gel produced by the above example, gelatin and a pectin gel cross-linked with diferulic acid which was prepared according to the teaching of French patent specification 2545101.

As can be seen from Figure 1, the gel according to the present invention had superior hardness compared to gelatin and the pectin gel, similar elasticity to gelatin and was less brittle than either of the other two gels.

Figures 2 and 3 respectively show the variation of elasticity and brittleness, hardness and adhesiveness with polysaccharide concentration of the gel (w/v).

EXAMPLE 2

Co-Gelling of Corn Bran and Wheat Bran Extracts

1. An extract of corn bran was prepared as in steps 1-4 of Example 1.

2. Wheat bran was macerated in hot water (70°C) and hot water soluble gums and starches removed by centrifugation at 2500rpm for 15 minutes discarding the supernatants.

3. The pellet of insoluble material was resuspended in hot water (80°C) and further centrifuged to remove soluble matter. This procedure was repeated until no more soluble matter was removed.

4. The remaining insoluble matter was suspended to 10% w/v in 2% KOH and stirred gently at 65-80°C for 2-3 hours, after which insoluble material was removed by centrifugation at 2500 rpm for 20 minutes.

5. The supernatant was neutralised with acid (hydrochloric or citric) and dialysed against running water for 2 days.

6. The extracts obtained from steps 1-5 and the corn bran extract obtained from steps 1-4 of Example 1 were mixed so as to give a solution containing wheat bran extract at 2.0% w/v and corn bran extract at 0.5% w/v. To 100ml of this mixture was added 1ml of 100 micrograms/ml horseradish peroxidase with mixing, followed by 0.5ml hydrogen peroxide at 40 micrograms H_2O_2/ml. After mixing the solution was left to set for 5-15 minutes at room temperature, for 1-2 minutes at 40°C or for less than one minute at 50°C.

In contrast, neither the 2.0% wheat bran nor the 0.5% corn bran extracts described above would form a firm gel when used alone.

EXAMPLE 3

Purification of Corn Bran Extract

An extract of corn bran prepared as in steps 1 - 4 of Example 1 was purified as follows:

1. Neutralisation

   The extract was neutralised with hydrochloric acid to pH 6 -6.5 and diluted to about 1.5% dry matter with water.

2. Salt Removal (Optional)

   The extract was desalted by dialysis against running water for 3 days. Alternatively this step may involve tangential flow ultrafiltration.

3. Separation

   The extract was then passed through a column containing activated carbon at a rate of 2 - 4 bed volumes per hour until the capacity of the column was exhausted. An eluate which was substantially free of mono and oligosaccharides, free ferulic and diferulic acids, and other organic compounds which contribute to colour and odour, was obtained.

4. Concentration

   The eluate was concentrated by precipitation with ammonium sulphate (other precipitating reagents such as ethanol, IMS propan-2-ol or methanol could have been used). Alternatively the concentration could have been carried out by drying (spray or vacuum rotary drying) and redissolving of the eluate.

5. Precipitation

   The redissolved precipitate produced in stage 4 was subjected to alcohol precipitation by adding 2.8 volume of alcohol.

6. Peroxide Treatment

The redissolved precipitate was added to water to produce a gelling medium of hemicellulosic concentration between 0.05 and 3.0% w/v. 30 - 100 micromoles of peroxide per gram of the polysaccharide and 100 - 200 microgram of peroxidase enzyme were added to the medium.

The above purification process could similarly be applied to a wheatbran extract.

CLAIMS:

1. A method of producing a gel material, which method comprises:
   (a) providing an aqueous soluble hemicellulosic starting medium which is substantially free of glucans and is obtainable from testaceous plant material;
   (b) extracting said starting medium with a non-acidic aqueous reagent; and
   (c) reacting the extracted material with an oxidising system comprising at least one peroxide, together with at least one oxygenase.

2. A method according to claim 1, wherein the starting medium is prepared from waste testaceous plant material containing at least about 20% of arabinoxylan and/or glucuronoarabinoxylan.

3. A method according to claim 2, wherein said waste material comprises cereal husk or bran, or legumes.

4. A method according to claim 3, wherein the cereal husk or bran comprises one or more of maize, barley, wheat, rice or oats, or malt or malt culms.

5. A method according to any of claims 1 to 4, wherein the starting medium is in a substantially ground form having a particle size of not more than about 100 microns.

6. A method according to claim 5, wherein the ground material is air classified or sieved to remove starch.

7. A method according to any of claims 1 to 6, which comprises starch removal by enzyme treatment.

8. A method according to claim 7, wherein the enzyme treatment comprises treatment with alpha and/or beta-amylase.

9. A method according to any of claims 1 to 8, wherein the glucans have been removed from the hemicellulosic starting medium by enzyme digestion with carbohydrase enzyme material.

10. A method according to any of claims 1 to 9, wherein the extraction comprises treatment with hot water or a weak alkali of less than 0.5%.

11. A method according to claim 10, wherein the alkali is NaOH or KOH.

12. A method according to claim 10 or 11, wherein the alkali is used in an amount of 0.1 to 10% for times of from 20 minutes to 5 hours.

13. A method according to claim 12, wherein the alkali is used in an amount of 0.5 to 2.5% for about 2 hours.

14. A method according to any of claims 10 to 13, wherein the alkaline extraction is carried out at a temperature of from 30 to 100°C.

15. A method according to claim 10, wherein the hot water extraction is carried out at a temperature of 50 to 80°C for 0.5 to 2 hours.

16. A method according to any of claims 1 to 15, which comprises acid treatment following extraction.

17. A method according to claim 16, wherein the acid comprises hydrochloric, sulphuric, acetic or citric acid.

18. A method according to any of claims 1 to 17, wherein the resulting gel is dried.

19. A method according to any of claims 1 to 18, wherein the hemicellulosic starting medium is substantially purified by precipitation with an alcohol.

20. A method according to claim 19, wherein the alcohol is methanol, ethanol or iso-propanol.

21. A method according to claim 19 or 20, wherein the alcohol is added in amounts of from 1.5 to 3.5 volumes.

22. A method according to any of claims 1 to 21, wherein the hemicellulosic starting medium is purified by ion-exchange treatment.

23. A method according to any of claims 1 to 22, wherein the hemicellulosic starting medium is purified by passage through an activated carbon column.

24. A method according to claim 22 or 23, wherein the eluate is concentrated by spray or vacuum rotary drying.

25. A method according to any of claims 1 to 24, wherein the extracted hemicellulosic starting medium is concentrated by precipitation with ammonium sulphate, methanol, ethanol or iso-propanol.

26. A method according to any of claims 1 to 25, which comprises dialysis or tangential flow ultrafiltration to lower the salt content of the hemicellulosic starting medium.

27. A method according to any of claims 1 to 26, wherein the oxygenase comprises a peroxidase.

28. A method according to claim 27, wherein the peroxidase comprises horseradish peroxidase.

29. A method according to claims 27 or 28, wherein the peroxidase is used in an amount of 1 to 100 micrograms per gram of substrate.

30. A method according to any of claims 27 to 29, wherein the peroxide is used in an amount of about one tenth of the amount of peroxidase.

31. A method according to any of claims 1 to 30, wherein the peroxide comprises hydrogen peroxide.

32. A method according to any of claims 1 to 31, which comprises addition of polyvalent metal cations prior to the peroxide treatment.

33. A method according to claim 32 wherein the metal cations comprise one or more of Ca^{2+}, Cu^{2+}, Zn^{2+}, Fe^{3+} or Al^{3+}.

34. A method substantially as hereinbefore described with reference to the examples.

Patents Act 1977
Examiner's report to the Comptroller under Section 17 (The Search Report)

Application number
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Relevant Technical fields

(i) UK Cl (Edition K) C3U (UDB, UDE)

(ii) Int Cl (Edition 5) C08B

Search Examiner
K MACDONALD

Databases (see over)

(i) UK Patent Office

(ii) ONLINE DATABASE: WPI

Date of Search
3 11 92

Documents considered relevant following a search in respect of claims 1 TO 34

Category (see over)	Identity of document and relevant passages	Relevant to claim(s)
Y	EP 0124439 A2 (INSTITUT NATIONAL DE LA RECHERCHE) Claims 1 and 2	at least Claim 1
Y	Nomenclature Committee of the International Union of Biochemistry "Enzyme Nomeuclature" published 1984, Academic Press Inc, pages 317-319 see eg Nos. 3.2.1.58, 3.2.1.59, 3.2.1.70, 3.2.1.74, 3.2.1.71, 3.2.1.75	at least Claim 1
Y	R L Whistler and C L Smart "Polysaccharide Chemistry", published 1953, Academic Press Inc. pages 120-122	at least Claim 1

Category	Identity of document and relevant passages	Relevant to claim(s)

Categories of documents

X: Document indicating lack of novelty or of inventive step.

Y: Document indicating lack of inventive step if combined with one or more other documents of the same category.

A: Document indicating technological background and/or state of the art.

P: Document published on or after the declared priority date but before the filing date of the present application.

E: Patent document published on or after, but with priority date earlier than, the filing date of the present application.

&: Member of the same patent family, corresponding document.

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